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(57) Abstract

Process for recovery of a β-lactam antibiotic from a mixture containing β-lactam antibiotic and D-phenyl glycine (FG) in solution, with the mixture being brought to a pH between (3) and (8) at a concentration such that FG remains in solution, the solid β-lactam antibiotic obtained being recovered and the remaining liquid being subjected to a concentrating step in which a slurry with solid β-lactam antibiotic and solid FG develops, the slurry is brought to a pH at which the β-lactam antibiotic dissolves, FG is separated as a solid and the β-lactam antibiotic present in the mother liquor is at least partially utilized. Preferably, use is made of an initial mixture substantially containing β-lactam antibiotic and FG originating from an enzymatic acylation reaction in which the corresponding β-lactam nucleus, in particular 6-aminopenicillanic acid, 7-aminodesacetoxycephalosporanic acid and 7-amino-3-chloro-cef-3-em-4-carboxylic acid, is acylated with a D-phenyl glycine derivative.

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PROCESS FOR RECOVERY OF A β-LACTAM ANTIBIOTIC

The invention relates to a process for recovery of a β-lactam antibiotic from a mixture substantially containing β-lactam antibiotic and D-phenyl glycine (FG) in solution, with the mixture being brought to a pH between 3 and 8 at a concentration such that FG remains in solution, the solid β-lactam antibiotic obtained being recovered and the remaining liquid being subjected to a concentration step in which a slurry with solid β lactam antibiotic and solid FG develops, the slurry is brought to a pH at which the β-lactam antibiotic dissolves, FG is separated as a solid and the β-lactam antibiotic present in the mother liquor is at least partially utilized.

In general, in the preparation of β-lactam antibiotics involving the acylation of a β-lactam nucleus with a D-phenyl glycine derivative, the β-lactam antibiotic is difficult to recover and the reaction mixture is difficult to work up. Working up often involves substantial losses of valuable components, in particular the β-lactam antibiotic, partly in the form of solubility losses and partly because of degradation as a result of the limited stability of β-lactam antibiotics.

The invention provides a new concept for recovery of β-lactam antibiotics whereby, in a simple process that can be applied on an industrial scale, the losses of β-lactam antibiotics are strongly reduced and also valuable D-phenyl glycine is recovered.

During the acylation reaction of the lactam nucleus with a suitable acylation agent, in particular an enzymatic acylation reaction with for example an amide of D-phenyl glycine, for example D-phenyl glycine amide (PGA) or an ester of D-phenyl glycine, for example the methyl ester of D-phenyl glycine (PGM), the acylation agent hydrolyzes with the β-lactam antibiotic to form D-phenyl glycine (PG).

The mixtures obtained after an acylation reaction may contain, besides the β-lactam antibiotic and PG, for example as-yet unconverted β-lactam nucleus and/or acylation agent, for example PGA or PGM. It has been found that the exact compositions of the mixtures that may be applied in the process according to the invention are not particularly critical. Mixtures that may suitably be applied in the process according to the invention are preferably mixtures containing 10-1500 mM, in particular 50-1000 mM β-lactam antibiotic; 0-1500 mM, in particular 0-1000 mM PG, 0-1000 mM, in particular 0-200 mM β-lactam nucleus and 0-1000, in particular 0-400 mM D-phenyl glycine derivative.

If necessary, a mixture containing β-lactam antibiotic and PG is brought to such concentration and pH that all components, in particular the components mentioned, optionally without the β-lactam antibiotic, are dissolved. To that end, the pH may be chosen to be either high, for example between 6.5 and 11, preferably between 7 and 9.5, or low, for example between 0 and 3, in particular between 0.3 and 2. When the process is to be carried out on a large scale, a more or less continuous working-up process is preferably opted for. A continuous dissolving process allows a shorter residence time at relatively high or low pH. If desired, any solid components still present can be

separated out by for example filtration or ultrafiltration.

According to the invention, the mixture, which may still contain solid β-lactam antibiotic, is first brought to a pH between 3 and 8, preferably between 4 and 7, with measures being taken, for example adding water, to ensure that the concentration of the reactants, in particular FG, is such that the reactants, with the exception of the β-lactam antibiotic, remain in solution, whether supersaturated or not. Preferably, the concentration is so chosen that the FG is supersaturated in the solution after the solution has been brought to a pH between 3 and 8. The temperature is not particularly critical and is between for example -5 and 45°C, in particular between 0 and 25°C. If the FG concentration in the mixture is relatively high, a temperature preferably between 0 and 10°C is maintained. This is because it has surprisingly been found that FG can be heavily supersaturated under these condition, without FG precipitating. This enabled relatively high concentrations to be maintained, so that a smaller volume is required and less β-lactam antibiotic remains in solution. Consequently, the β-lactam antibiotic substantially precipitates as a solid whereupon it can be recovered.

It has been found, however, that the amount of β-lactam antibiotic present in the mother liquor after recovery of the solid β-lactam antibiotic still is relatively high. The applicant has now found a method enabling this liquor to be worked up further and enabling the yield of β-lactam antibiotic to be increased further. According to the invention, the liquid is subjected to concentration. To prevent

degradation of the β-lactam antibiotic and discolouration of the β-lactam antibiotic and the FG crystals, a relatively low temperature should be chosen and the concentration step should last a relatively short period whilst on the other hand a relatively high temperature and a relatively long, gradual concentration procedure should be chosen for obtaining a not-too-viscous slurry and large FG crystals that can readily be separated. Surprisingly, it has been found possible to conduct a concentration step under such conditions that degradation of the β-lactam antibiotic is drastically reduced and yet the FG crystals are relatively large and can readily be separated.

The temperature at which the concentration is effected may be between for example 10 and 80°C, preferably between 20 and 60°C, in particular between 25 and 55°C. The duration of the concentration is between for example 10 min. and 24 hours, preferably between 0.5 and 10 hours, in particular between 1 and 5 hours. The temperature at which the concentration is effected and the duration of the concentration are so chosen that, as a rule, a relatively shorter duration is chosen in combination with a higher temperature, and conversely.

Concentration may be effected by for example evaporation at reduced pressure or by nanofiltration. Evaporation may be effected in for example a thin-film evaporator. It has been found that the wall is easy to keep clean and that little degradation of the β-lactam antibiotic took place. Also, it has been found that FG crystals of better quality are obtained when evaporation is carried out gradually by for example passing the liquid suspension to be evaporated through the evaporator at a high flow

rate, in which process the composition of the liquid changes gradually. A second possible embodiment of the concentration is an evaporator-crystallizer, for example a bypass evaporator or a number of cascaded bypass evaporators.

It has been found that concentration can also be very well effected by means of nanofiltration. Surprisingly, it has been found that the flux through the membrane remains relatively high even though crystallization occurs during nanofiltration. Membranes that may suitably be applied in concentration through nanofiltration are for example SelRO MPT-10 (Membrane Products Kiryat Weizmann), WFN0505 (Stork Friesland) and Nanomax-50 (Millipore).

The concentration factor, i.e. the volume ratio before and after concentration, may vary between wide limits and preferably is between 1 and 30, in particular between 3 and 20. The higher the concentration factor, the higher the efficiency will be. Preferably, the concentration factor is chosen so that salts developing throughout the process remain dissolved. Those skilled in the art can readily determine the optimum concentration factor in their particular case.

The slurry obtained after the concentration step preferably is first subjected to separation into a clear liquid stream and a concentrated slurry or into a clear liquid stream and a solid. Separation may be effected with any separation apparatus, for example a centrifuge or a filter. Since the solids need not necessarily be separated as solids, it is preferred for a decanter to be used. The clear liquid stream may for example be discharged; this affords a simple manner of preventing degradation products and salts from building up. Since this discharge stream is relatively small,

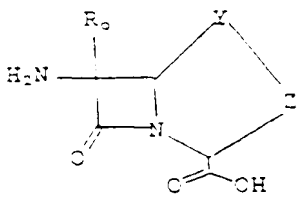
only little β-lactam antibiotic is lost with it because of solubility losses.

During concentration, both FG and β-lactam antibiotic are formed as a solid. It has been found that the solid FG formed in the concentration step can readily be filtered. In consequence, due in part to the fact that the concentration of β-lactam antibiotic now is relatively low, it was now found to be possible to recover FG by bringing the slurry to a pH at which the β-lactam antibiotic dissolves and FG does not, for example a pH between 6.5 and 11, preferably between 7 and 9.5, or between 0 and 3, preferably between 0.3 and 2, and next separating FG as a solid by for example filtration or centrifuging. Since the solid can now be separated rapidly, the separation process involves relatively little degradation of the β-lactam antibiotic in spite of the high, or low, pH. If desired, the filtrate can subsequently be returned to the process or reused otherwise.

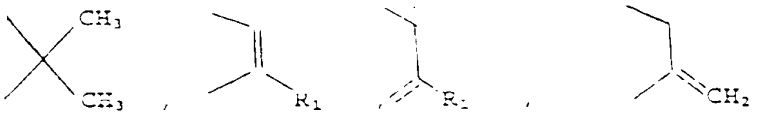
The β-lactam antibiotic in the aforementioned filtrate can be recovered by for example crystallization via a pH shift. Another possibility is to return the filtrate as such to the mixture containing dissolved β-lactam antibiotic and FG.

The process according to the invention can suitably be applied in the preparation of such β-lactam antibiotics as have a phenyl glycine side chain, for example cephalexin, ampicillin, cephaclor, pivampicillin, becampicillin, talampicillin and cefaloglycine.

Any β-lactam nucleus can in principle be used, in particular a β-lactam nucleus with the general formula (1)



where R_o represents H or an alkoxy group having 1-3 C atoms; >Y represents CH_2, O, S or an oxidized form of sulphur; and >Z represents



where R_1 represents for example H, OH, halogen, an alkoxy group having 1-5 C atoms, an alkyl group having 1-5 C atoms, a cycloalkyl group having 4-8 C atoms, an aryl or a heteroaryl group having 6-10 C atoms in which the groups may or may not be substituted with for example an alkyl, an aryl, a carboxy or an alkoxy group having 1-8 C atoms; and where the carboxylic acid group may be an ester group if so desired.

Suitable examples of β-lactam nuclei that may be employed in the process according to the invention are penicillin derivatives, for example 6-aminopenicillanic acid (6-APA) and cephalosporanic acid derivatives, for example a 7-aminocephalosporanic acid with or without a substituent at the 3-site, for example 7-aminocephalosporanic acid (7-ACA), 7-aminodesacetoxycephalosporanic acid (7-ADCA) and 7-amino-3-chloro-cef-3-em-4-carboxylic acid (7-ACCA).

In principle, any enzyme that is suitable as a catalyst in the coupling reaction can be used as the enzyme. Such enzymes include the enzymes

collectively referred to as penicillin amidase or penicillin acylase. Such enzymes are described in for example J.G. Shewale et al., Process Biochemistry, August 1989, pp. 146-154 and in J.G. Shewale et all, Process Biochemistry International, June 1990, pp. 97-103. Examples of suitable enzymes are enzymes derived from Acetobacter, in particular Acetobacter pasteurianum, Aeromonas, Alcaligenes, in particular Alcaligenes faecalis, Aphanocladium, Bacillus sp., in particular Bacillus megaterium, Cephalosporium, Escherichia, in particular Escherichia coli, Flavobacterium, Fusarium, in particular Fusarium oxysporum and Fusarium Solani, Kluyvera, Mycoplana, Protaminobacter, Proteus, in particular Proteus rettgari, Pseudomonas and Xanthomonas, in particular Xanthomonas citrii.

Preferably an immobilized enzyme is used, since the enzyme can be easily isolated and re-used then. A suitable immobilization technology is described for instance in EP-A-222462. Another suitable technology consists in immobilizing the Penicillin G acylase on a carrier which contains a gelating agent, for instance gelatin, and a polymer with free amino groups, for instance alginate amine, chitosan or polyethylene imine. In addition, enzymes may also be utilized as a crystalline substance (CLEC's™).

Particularly suitable enzymes among the immobilized enzymes that are commercially available are the Escherichia coli enzyme from Boehringer Mannheim GmbH, which is commercially available under the name Enzygel®, the immobilized Penicillin-G acylase from Recordati and the immobilized Penicillin-G acylase from Pharma Biotechnology Hannover.

In the (enzymatic) acylation reaction, the

acylation agent may be for instance a D-phenyl glycine in activated form, preferably a (primary, secondary or tertiary) amide or salt thereof, or a lower alkyl (1-4C) ester, for instance a methyl ester.

The temperature at which the enzymatic acylation reaction is effected usually is below 40°C, preferably between -5 and 35°C. The pH at which the enzymatic acylation reaction is effected usually is between 5.5 and 9.5, preferably between 6.0 and 9.0.

The reaction preferably is stopped almost completely when maximum conversion has virtually been achieved. A suitable embodiment for stopping the reaction is to lower the pH, preferably to a value between 4.0 and 6.3, in particular between 4.5 and 5.7. Another suitable embodiment is to lower the temperature of the reaction mixture on attaining the maximum conversion. A combination of the two embodiments is possible also.

Once the reaction has been stopped on attaining the maximum conversion, the reaction mixture usually is present in the form of a suspension comprising a plurality of solids, for example the antibiotic, D-phenyl glycine and, possibly, immobilized enzyme. The immobilized enzyme preferably is recovered in the interest of process economics. This can suitably be accomplished by for example filtering the reaction mixture on a sieve, while stirring, the stirrer's direction of rotation being chosen so that the suspension is pumped upwards at the centre of the stirrer. Subsequently, valuable components such as the antibiotic and FG can be recovered by the process according to the invention, with the solid components, possibly apart from solid antibiotic, being dissolved first, by means of for example a pH shift.

The pH may be lowered in several ways in the framework of the invention, for instance by adding an acid to the mixture. Suitable acids are for example mineral acids, in particular sulphuric acid, hydrochloric acid or nitric acid. Preferably, hydrochloric acid is used. The pH can be raised by for example adding a base to the mixture. Suitable bases are for example inorganic bases, in particular ammonium hydroxide, potassium hydroxide or sodium hydroxide. Preferably, ammonium hydroxide is used.

In practice, the enzymatic acylation reaction and the working-up of the reaction mixture are usually effected in water. If desired, the reaction mixture may also contain an organic solvent or a mixture of organic solvents, preferably less than 30 vol.%. Examples of suitable organic solvents that can be used are alcohols having 1-7 C atoms, for example a monoalcohol, in particular methanol or ethanol; a diol, in particular ethylene glycol, or a triol, in particular glycerol.

The process according to the invention is particularly suited for being used in working up the reaction mixture obtained after the enzymatic acylation reaction in which 6-APA is acylated with an amide of D-phenyl glycine, for example PGA, or an ester of D-phenyl glycine, for example PGM.

In a preferred embodiment of the process, measures are taken to ensure that the concentration of dissolved 6-APA in the reaction mixture is kept relatively low so that a higher conversion can be achieved than when the concentration of dissolved 6-APA is chosen to be as high as possible.
Moreover, it has been found that the stirrability of the reaction mixture is significantly higher when the concentration of dissolved 6-APA is kept low.

In the present context, 'conversion' refers to the molar ratio of the ampicillin formed and the amount of 6-APA used. The concentration of dissolved 6-APA is expressed as the amount of 6-APA in moles per kg of the reaction mixture; the total concentration, dissolved and undissolved, of 6-APA is expressed as the amount of 6-APA plus ampicillin in moles per kg of the total reaction mixture; the total reaction mixture may contain, besides the solution, a number of solids, for example 6-APA, ampicillin, phenyl glycine and immobilized enzyme.

The molar ratio of acylation agent and 6-APA, i.e. the total amount of phenyl glycine derivative added, divided by the total amount of 6-APA added, expressed in moles, is preferably less than 2.5. It is preferred for the molar ratio to be between 1.0 and 2.0, in particular between 1.2 and 1.8.

The enzymatic acylation reaction is preferably carried out as a batch process. If desired, the reaction can also be carried out continuously, with in-line control of the concentration of dissolved 6-APA.

The total concentration of 6-APA plus ampicillin (in dissolved and undissolved form) in the reaction mixture preferably is chosen to be higher than 250 mM, more preferably higher than 300 mM, in particular higher than 350 mM.

In this preferred embodiment, because of the increased instability of 6-APA at higher concentrations of dissolved 6-APA, the concentration of dissolved 6-APA during the preparation of ampicillin is preferably kept below 300 mM, in particular below 250 mM. At a higher concentration of the acylation agent, the concentration of dissolved 6-APA may optionally chosen to be higher than at a lower concentration. This

is because the rate of reaction is higher at higher concentrations of the acylation agent, so that 6-APA is dissolved in a high concentration for only a relatively short period.

The concentration of 6-APA dissolved in the reaction mixture can be kept low in various ways. One possibility of keeping the concentration of dissolved 6-APA low is to initially feed only a portion of the total amount of 6-APA and to meter in the balance during the reaction. A drawback of this is that in that case 6-APA needs to be metered in solid form, which presents practical problems. Therefore, it is preferred in a batch process for the total amount of 6-APA to be supplied at the start of the reaction, whereupon, during the enzymatic acylation reaction, the concentration of 6-APA in the reaction mixture will decrease and the concentration of ampicillin will increase. A suitable method of achieving a low concentration of dissolved 6-APA is for example to keep the pH at a lower value than that at which maximum solubility of the reactants is achieved. A particularly suitable method of keeping the dissolved 6-APA concentration low is for example to ensure that the concentration of the phenyl glycine derivative is kept low, for example by metering in the phenyl glycine derivative partly in the course of the reaction.

In this context, it has been found that, if the concentration of the phenyl glycine derivative is kept low, only little 6-APA is dissolved so that the dissolved 6-APA concentration can be controlled by metering the phenyl glycine derivative.

A particularly suitable embodiment is obtained when FGA is added in the form of one of its salts, preferably the salt of FGA and a mineral acid, for example FGA.HCl, FGA.1/2H_2SO_4 and FGA.HNO_3. In this

manner it is possible to readily ensure optimum metering of the FGA by keeping the pH constant. Preferably, FGA.1/2H_2SO_4 is used inasmuch as this salt possesses extremely high solubility.

In the framework of the present invention the various components may be present in the reaction mixture in the free form or as salts. The pH values mentioned are in all cases the pH values measured at room temperature.

The invention will be further elucidated by means of the following examples, without however being restricted thereto.

Abbreviations:

AMPI = ampicillin
AMPI.3H_2O = ampicillin trihydrate
6-APA = 6-amino-penicillanic acid
FGA = D-phenyl glycine amide
FG = D-phenyl glycine
FGHM = D-p-hydroxyphenyl glycine methyl ester

Assemblase™ is an immobilized Escherichia coli penicillin acylase from E. coli ATCC 11105 as described in WO-A-97/04086. The immobilization is effected as set out in EP-A-222462, with gelatin and chitosan being used as gelating agents and glutaraldehyde as crosslinking agent.

The ultimate activity of the Escherichia coli penicillin acylase is determined by the amount of enzyme added to the activated spherules and amounted to 3 ASU/g of dry weight, 1 ASU (Amoxicillin Synthesis Unit) being defined as the amount of enzyme capable of producing 1 g of Amoxicillin.3H_2O from 6-APA and FGHM per hour (at 20°C; 6.5% 6-APA and 6.5% FGHM).

Example I

Preparation of FGA.1/H_2SO_4 solution

301.6 g of FGA (2.00 mole) were suspended in 650 g of water at T = 5°C. 102.1 g of 96-% H_2SO_4 (1.00 mole) were added drop-wise in 1 hour while stirring, with the temperature being kept at T < 25°C by means of cooling.

Example II

Enzymatic synthesis of ampicillin

An enzyme reactor (1.5 l, diameter 11 cm), fitted with a 175 µm mesh sieve bottom, was filled with 300 g of net-wet assemblase™ (the term net-wet refers to the mass of the enzyme obtained on separating the enzyme from an enzyme slurry with the aid of a glass filter).

A preparation reactor (1.2 l) was filled with 131.6 g of 6-APA (0.600 mole), 30.2 g of FGA ((0.200 mole) and 400 ml of water (T = 10°C). This mixture was stirred for 15 minutes at T = 10°C and then at t = 0 transferred into the enzyme reactor with the aid of 100 ml of water (T = 10°C).

The stirrer in the enzyme reactor was switched on at t = 0. The temperature was kept at 10°C all the time. 423.7 g of FGA.½H_2SO_4 solution (0,800 mole) were added at a constant rate over a period of 233 minutes. The pH was kept constant at approx. 6.3 by titration with 6N H_2SO_4. At t = 570 minutes the amount of AMPI was maximum and the pH was reduced to 5.0 by adding 6N H_2SO_4. The enzyme reactor now contained:

575 mmole AMPI

15 mmole 6-APA

50 mmole FGA
365 mmole FG

Example III

Separation of AMPI/FG slurry from enzyme reactor

The AMPI/FG slurry prepared as described in Example II was removed from the enzyme reactor via the sieve bottom by means of stirred filtration. This was done using a pitched-blade stirrer, which was positioned at 0.5 cm over the sieve. Stirring was in upward direction at approx. 500 rpm. The reactor was flushed with 10 portions of 250 ml of water (T = 10°C). The wash waters, too, were removed via the sieve bottom by means of stirred filtration.

The AMPI/FG slurry and all was waters separated from the reactor were combined (T = 10°C). The resulting AMPI/FG slurry so obtained contained > 99.8% of the total amount of AMPI produced in the enzyme reactor and > 99.5% of the total amount of FG produced.

After this stirred filtration, > 99.5% of the Assemblase™ was present in the enzyme reactor.

Example IV

Concentration of the ampicillin mother liquor by evaporation with the aid of a thin-film evaporator

A mother liquor from an AMPI crystallization step (amount: 6798 g, T = 5°C, pH = 6.0) contained 0.06% (17 mmole) 6-APA, 0.10% (45 mmole) FGA, 0.53% (104 mmole) AMPI and 0.96% (430 mmole) FG.

This mother liquor was concentrated by evaporation with the aid of a thin-film evaporator. The feed to the thin-film evaporator was supplied from a storage vessel, and the product from the thin-film evaporator was returned to the same storage vessel. The

storage vessel was stirred.

The wall temperature of the thin-film evaporator was adjusted to t = 65°C and the pressure in the thin-film evaporator was adjusted to P = 80 mbar.

Circulation was started at t = 0 (storage vessel ⇒ thin film evaporator ⇒ storage vessel; flow = 41.6 kg/hour).

The temperature of the liquid in the storage vessel was maintained at T = 40°C.

Evaporation was stopped at t = 257 minutes. A total of 5655 g of condensate had been collected by then. The contents of the thin-film evaporator and the storage vessel were circulated for another 2 hours while the temperature was lowered linearly from T = 40°C to T = 3°C. Thereafter, the concentrate (1084 g) was drained. The thin-film evaporator and the storage vessel were flushed first with 250 ml and next with 2000 ml of water (T = 10°C). The condensate and the 1st wash water were combined. The 2nd wash water contained negligible amounts of AMPI and FG.

Example V

Return of concentrated ampicillin mother liquor to the ampicillin process as recycle; separation of FG.

The combined concentrate + 1st wash water (Example IV) was filtered on a glass filter (diameter 10 cm, filtration time 10 minutes). The solid product was rewashed with 25 ml of water (T = 5°C). Yield: 210 g of AMPI/FG wet cake. The mother liquor (1155 g) was discharged.

The AMPI/FG wet cake was quantitatively transferred to a stirred reactor with the aid of 400 g of water. The slurry was stirred for 1 hour at T = 5°C. Subsequently, 33.0 g of 6N HCl were added in 4 minutes

at T = 5°C. After stirring for another 5 minutes, the slurry was filtered on a glass filter (diameter 13 cm, filtration time 5 minutes). The FG wet cake was washed with 120 ml of water (T = 5°C) and dried. Yield = 36.7 g of FG. The mother liquor and the wash water were combined (T = 5°C) and after 2 minutes added to the acid solution of Example VI (for 30 minutes).

Example VI

Recrystallization of ampicillin, including recycle of the concentrated ampicillin mother liquor

Recrystallization was effected in a rig consisting, in the order as stated, of a storage vessel (4 l), a dissolving vessel (0.25 l), a filter fitted with a Seitz filter plate, and a crystallization vessel (7 l). All vessels were provided with a stirrer, a pH electrode and a thermometer.

200 ml of water (T = 5°C) containing 5.0 g of AMPI.$3H_2O$ as nucleus were added to the crystallization vessel. The pH was brought to pH = 7 by means of 2N NaOH solution.

The AMPI/FG slurry which had been isolated as described in Example III was quantitatively transferred to the storage vessel and cooled to T = 2°C while being stirred. After the entire loop had been brought T = 2°C, the dissolving vessel was filled from the storage vessel.

At t = 0 the pH in the dissolving vessel was adjusted to pH = 1.1 with the aid of 6N HCl, whereupon the pH was maintained at 1.1 (titration with the aid of 6N HCl). Next the contents of the storage vessel were added to the dissolving vessel in 1 hour while the contents of the dissolving vessel were metered into the crystallization vessel, the level in the dissolving

vessel being kept constant. The temperature in the crystallization vessel was maintained at T = 5°C. The pH in this vessel was maintained at pH = 7.0 by titrating with 2N NaOH solution. Also, the combined mother liquor and wash water, which had been prepared as described in Example V, were added to the dissolving vessel at a constant rate from t = 15 minutes to t = 45 minutes.

At t = 60 minutes the storage vessel was empty and a total of 300 ml of 6N HCl solution had been added to the dissolving vessel. The storage vessel was flushed to the dissolving vessel with 450 g of water (T = 5°C). Titration with 6N HCl was stopped. The dissolving vessel was flushed to the crystallization vessel with 50 g of water (T = 5°C). The pH in the crystallization vessel was reduced to 6.0 with the aid of 6N HCl. After stirring for 1 hour, the slurry in the crystallization vessel was filtered on a glass filter and the wet cake was washed with 2 x 175 ml of water (T = 5°C) and dried. Yield:

227 g of AMPI.$3H_2O$ (incl. nuclei), so
222 g of AMPI.$3H_2O$ (excl. nuclei), that is,
92% AMPI.$3H_2O$ relative to 600 mmoles of 6-APA.

C L A I M S

1. Process for recovery of a β-lactam antibiotic from a mixture containing the β-lactam antibiotic and D-phenyl glycine (FG) in solution, with the mixture being brought to a pH between 3 and 8 at a concentration such that FG remains in solution, the solid β-lactam antibiotic obtained being recovered and the remaining liquid being subjected to a concentration step in which a slurry with solid β-lactam antibiotic and solid FG develops, the slurry is brought to a pH at which the β-lactam antibiotic dissolves, FG is separated as a solid and the β-lactam antibiotic present in the mother liquor is at least partially utilized.
2. Process according to Claim 1, in which the concentration of the mixture is chosen so that the FG is supersaturated in the solution after the mixture has been brought to a pH between 3 and 8.
3. Process according to Claim 1 or 2, in which the β-lactam antibiotic present in the mother liquor is at least partially recovered.
4. Process according to any one of Claims 1-3, in which the mother liquor is returned and combined with the mixture.
5. Process according to any one of Claims 1-4, in which concentration is effected for 0.5-10 hours at a temperature between 20 and 60°C.
6. Process according to Claim 5, in which concentration is effected for 1-5 hours at a temperature between 25 and 55°C.
7. Process according to any one of Claims 1-6, in which concentration is effected in a thin-film

evaporator.

8. Process according to any one of Claims 1-7, in which the concentration factor during concentration is between 3 and 20.

9. Process according to any one of Claims 1-8, in which the slurry is first subjected to separation into a liquid stream and a concentrated slurry or solid.

10. Process according to Claim 9, in which the separation is effected in a decanter.

11. Process according to any one of Claims 1-10, in which the initial mixture, which substantially contains β-lactam antibiotic and FG, originates from an enzymatic acylation reaction in which the corresponding β-lactam nucleus is acylated with a D-phenyl glycine derivative.

12. Process according to Claim 11, in which the mixture obtained after the acylation reaction is first subjected to a pH change to a pH between 6.5 and 11 or a pH between 0 and 3.

13. Process according to Claim 12, in which the mixture is subjected to a pH decrease to a pH between 0.3 and 2.

14. Process according to any one of Claims 1-13, in which the mixture contains 10-1500 mM β-lactam antibiotic, 0-1500 mM FG, 0-1000 D-phenyl glycine derivative and 0-1000 mM β-lactam nucleus.

15. Process according to any one of Claims 1-14, in which the β-lactam nucleus is 6-aminopenicillanic acid (6-APA) and the β-lactam antibiotic is ampicillin.

16. Process according to any one of Claims 1-14, in which the β-lactam nucleus is 7-aminodesacetoxycephalosporanic acid (7-ADCA) and

the β-lactam antibiotic is cephalexin.

17. Process according to any one of Claims 1-14, in which the β-lactam nucleus is 7-amino-3-chloro-cef-3-em-4-carboxylic acid (7-ACCA) and the β-lactam antibiotic is cefaclor.

INTERNATIONAL SEARCH REPORT

Inte | Application No
PCT/NL 98/00538

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07D499/18 C07D501/12 C12P37/04 C12P35/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07D C12P A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 97 22610 A (CHEMFERM V.O.F.) 26 June 1997 see page 3, line 7 - page 4, line 19; claims	1-17
A	WO 96 30376 A (CHEMFERM V.O.F.) 3 October 1996 see the whole document	1-17
A	WO 96 23796 A (CHEMFERM V.O.F.) 8 August 1996 see the whole document	1-17
A	WO 95 03420 A (DSM N.V.) 2 February 1995 see the whole document	1-17

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents:

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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INTERNATIONAL SEARCH REPORT

Inte	Application No
	PCT/NL 98/00538

C (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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